Plant Physiol. (1982) 69, 771–775
0032-0889/82/69/0771/05/$00.50/0

Control of Leaf Expansion by Nitrogen Nutrition in Sunflower Plants[1]

ROLE OF HYDRAULIC CONDUCTIVITY AND TURGOR

Received for publication July 28, 1981 and in revised form November 23, 1981

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ABSTRACT

Nitrogen nutrition strongly affected the growth rate of young sunflower (*Helianthus annuus* L.) leaves. When plants were grown from seed on either of two levels of N availability, a 33% decrease in tissue N of expanding leaves was associated with a 75% overall inhibition of leaf growth. Almost all of the growth inhibition resulted from a depression of the daytime growth rate. Measurements of pressure-induced water flux through roots showed that N deficiency decreased root hydraulic conductivity by about half. Thus, N deficiency lowered the steady-state water potential of expanding leaves during the daytime when transpiration was occurring. As a result, N-deficient leaves were unable to maintain adequate turgor for growth in the daytime. N deficiency also decreased the hydraulic conductivity for water movement into expanding leaf cells in the absence of transpiration, but growth inhibition at night was much less than in the daytime. N nutrition had no detectable effects on plastic extensibility or the threshold turgor for growth.

A major consequence of N deficiency in plants is a decreased growth rate. Watson (26, 27) concluded that N deficiency limits growth primarily by limiting the rate of leaf area increase, rather than the rate of dry matter accumulation per unit leaf area. This conclusion is supported by numerous studies using both growth analysis and direct measurements of photosynthesis (1, 8, 9, 19). Much of this effect on leaf area can be ascribed to effects on cell expansion rather than cell division (18, 21), but there are no reports which might suggest an explanation.

Cell growth is commonly described by the relationship (11):

$$G = \frac{dl}{dt} = m(P - Y) \quad (1)$$

where G is linear extension rate (cm s^{-1}), l is length (cm), t is time (s), m is plastic extensibility (cm s^{-1} bar^{-1}), P is turgor pressure (bars), and Y is the wall yield threshold, or minimum turgor for growth (bars). In this paper, we discuss growth in terms of water uptake into the expanding cells. This quantity is related to G as follows:

$$J_g = \frac{dW}{dt} = \frac{dW}{dl}\frac{dl}{dt} = kG \quad (2)$$

where J_g is water flux associated with growth (cm^3 s^{-1}), W is water content of the leaf under study (cm^3), and k (=dW/dl, cm^2) is an empirically determined coefficient. The water uptake associated with growth can also be described by the transport equation:

$$J_g = kG = L'(\psi_o - \psi_w) \quad (3)$$

where L' is apparent hydraulic conductivity of the pathway between root surface and the expanding cells (cm^3 s^{-1} bar^{-1}), and ψ_o and ψ_w are water potentials of the soil and leaf, respectively (bars). Equations 1, 2, and 3 can be rearranged and combined (5, 12):

$$J_g = \frac{L'km\,(\psi_o - \psi_\pi - Y)}{L' + km} \quad (4)$$

where ψ_π is the leaf osmotic potential (bars). From these expressions, it is apparent that five plant properties (L', m, ψ_π, P, and Y) can control the growth rate. Here, we analyze the effects of N nutrition on sunflower leaf growth in terms of these five quantities.

MATERIALS AND METHODS

Plant Growth Conditions. Sunflower (*Helianthus annuus* L. cv. Russian Mammoth) plants were grown in 15-cm pots (two plants per pot) in a growth room with a 14-h daylength and a 30/22°C day/night temperature cycle. Quantum flux of 400 μE m^{-2} s^{-1} at plant height was provided by high-intensity fluorescent lamps (daylight-type). The soil mix was soil, peat, perlite, and vermiculite (4:1:1:1). A solution containing macronutrients plus iron was added to pots three times per week, and deionized water was added on all other days. In all cases, enough water or solution was added to insure substantial leaching. The nutrient solution contained either 5 mM NO_3^- (high N treatment) or 0.25 mM NO_3^- (low N treatment) as KNO_3 + $Ca(NO_3)_2$. Solutions were kept isoosmotic by additions of KCl + $CaCl_2$ to the low N solution. Water deficits were established by withholding water after the second true leaves were 5 cm long.

For experiments with pressure-induced flow through root systems, seedlings were transplanted soon after emergence into 0.35-liter cans containing soil mix. They were grown, one plant per container, as described above.

Leaf Water Potentials. Water potentials and osmotic potentials were measured by isopiestic thermocouple psychrometry (4) on duplicate tissue samples, one of which had been frozen on dry ice and thawed at room temperature. Turgor was calculated as ψ_w –

[1] Supported in part by a Training and Development grant from the Western Region, United States Department of Agriculture, Agricultural Research Service to J. W. R. and by National Science Foundation grant PCM 79-09790 to J. S. B.

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ψ_π.

Leaf Growth. Growth was measured when the second set of true leaves was 5 cm long, except as noted. The base of the expanding leaf was clamped to prevent movement, and the tip was clamped to a freely rotating wheel upon which a long pointer was mounted. With the leaf tip clipped to the edge of the wheel, the pointer moved along a calibrated scale as the leaf grew. The pointer amplified changes in leaf length about 10-fold so that differences in length of 0.05 mm were discernible. Rapid leaf 'stretching' was evident during the first 20 to 30 min because the weight of the pointer applied a small tension to the leaf, but thereafter increases in length were linear with time. Each data point is from at least 1 h of steady-state growth at rapid growth rates and proportionately longer at slow growth rates. After each run, leaf samples were taken for measurement of ψ_w and ψ_π.

In diurnal studies, leaf lengths were measured with a ruler at the beginning and end of each light period. Data are means of measurements on five plants.

Growth in a Water-Saturated Atmosphere. Plants were watered, then placed inside a urethane foam chest lined with water-saturated paper, and attached to the growth apparatus described above. Plants were allowed to equilibrate with the saturated atmosphere in darkness for about 2 h before growth measurements were begun. The position of the pointer of the growth apparatus was observed periodically through a small plastic window on the side of the chest to avoid opening the chest. Between readings the window was covered with aluminum foil to keep the plant in darkness. After establishment of a steady-state leaf extension rate for at least 1 h, the chest was opened and leaf samples were rapidly removed for ψ_w and ψ_π. Apparent hydraulic conductivity (whole plants) was derived from these measurements using equation 3 (assuming that $\psi_o = 0$).

Leaf and Plant Water Contents. Because N nutrition affects the water content of leaves per unit dry weight (21), the coefficient k in equation 2 should be expected to vary across treatments. The lengths, fresh weights, and dry weights of leaf blades from well-watered plants were measured during leaf expansion and used to calculate the following regressions of water content of blades against leaf length: for low N leaves, $W = 0.0082\ l^2 + 0.015$ ($r = 0.963$, $n = 26$), and for high N leaves, $W = 0.0117\ l^2 - 0.016$ ($r = 0.997$, $n = 24$). From these regressions, $k = dW/dl = 0.0164\ l$ for low N and $0.0234\ l$ for high N leaves. Thus, at $l = 5$ cm, $k = 0.082$ cm^2 for low N and 0.117 cm^2 for high N leaves.

A regression of shoot water content (W_s, cm^3) against W during growth yielded the relationship $W_s = 6.80\ W + 0.71$ ($r = 0.901$, $n = 35$) for pooled data of both treatments (well-watered plants). Thus, the water flux for growth of the entire shoot was 6.8 times greater than that for growth of one leaf.

Cell Wall Extensibility and Yield Threshold. Water was withheld from plants to initiate drying. Growth rates, water potentials, and osmotic potentials of leaves in the light were followed as stress progressed. The slope of the curve relating growth rate to turgor (dG/dP) was taken as a measure of extensibility (11). The intercept of the curve with the P-axis (minimum turgor for growth) was taken to be the yield threshold (11).

The extensibility and yield threshold were also determined under nighttime conditions. The same protocol was followed, except that stressed plants were transferred from light into darkness in a dry chest which was cooled to 22°C. After 2 h in darkness, steady-state growth was determined and leaf samples were taken as above. Growth rates changed within 1 h to the values characteristic of the 10-h night period, and readily reverted to daytime values when the plants were returned to the light.

Pressure-Induced Exudation. Plants were detopped just below the cotyledons and placed in a pressure chamber filled with nutrient solution. The assembly was sealed with the stem protruding through a silicone rubber insert in a rubber stopper clamped into place. Compressed air was bubbled into the nutrient solution from an inlet near the bottom. A small amount of air was continuously bled off by a valve near the top of the chamber to insure good aeration in the solution while pressure was held constant. The pressure was slowly increased to 3.5 bars and allowed to remain there for several min, then the cut stump was fitted with a glass tube to collect exudate. Once per min, exudate was removed with a syringe and its volume was determined. After flux was constant for 4 min, the pressure was decreased by 0.5 bar and the process was repeated. Osmotic potentials of exudate were determined psychrometrically (4).

Leaf Cell Size. Size of upper epidermal cells was determined by techniques described earlier (21).

Root Characteristics. Roots were gently washed free of soil to the extent possible and suspended in water. Root lengths of three plants from each treatment were estimated using Newman's (20) technique. Diameters of 35 randomly selected roots from each plant were measured under a dissecting microscope.

Plant Analyses. Total reduced N of expanding leaf blades was determined by titration following Kjeldahl digestion and Conway microdiffusion (22). NO_3^--N was determined by the procedure of Cataldo *et al.* (6).

RESULTS

Plant Characteristics. Most experiments were performed when leaves at the second node were 5 cm long. Because low N leaves expanded more slowly, this comparison required using plants of different ages. Although we did not determine cell numbers for the entire leaf, cell numbers in the upper epidermis were unaffected by N level at this growth stage (data not shown). Expanding low N and high N leaves contained 3.8% and 5.7% reduced N and 0.1% and 0.6% NO_3^--N, respectively, on a dry weight basis.

Nitrogen nutrition also affected root physical characteristics. For low N and high N plants, respectively, total root lengths were 17.1 ± 0.8 and 14.5 ± 0.2 m (means ± SE), and root diameters were 0.30 ± 0.02 and 0.33 ± 0.03 mm. Assuming cylindrical roots and a normal distribution of diameter over length, calculated surface areas were 0.016 and 0.015 m^2 for low N and high N plants, respectively.

Leaf Growth Rates. Long-term leaf enlargement was greatly slowed by N deficiency (Fig. 1, insert). Leaf length increased by about 1.2 cm/d at high N, but only about 0.3 cm/d at low N. Growth rates of low N leaves showed strong diurnal cycling, with the maximum rate at night (Fig. 1). At high N, growth differences between day and night were minimal, with a tendency toward a greater rate in the daytime (Fig. 1).

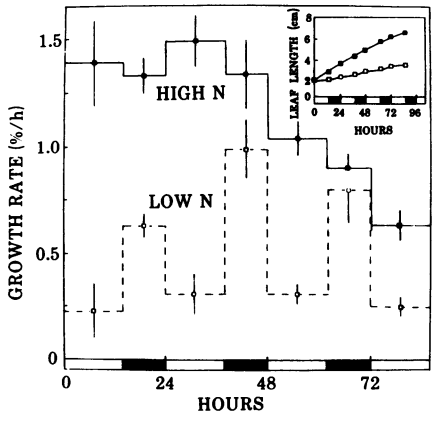

FIG. 1. Growth rates (% increase in length/h) of leaves of sunflower plants grown at two levels of N nutrition. Alternating light and dark bars indicate daytime and nighttime, respectively. Initial leaf length was 2 cm. Values shown ± SE. Insert, Overall leaf length *versus* time.

Table I. *Growth Rates, Water Potentials, and Turgors of Expanding Leaves of Sunflower Plants*

Plants at each N level were watered, divided into two groups, and placed in light or darkness. When growth rates had stabilized, samples were taken for water potentials and osmotic potentials. Values are shown ± SE.

Treatment	Growth Rate	Water Potential	Turgor
	%/h	*bars*	
Low N			
Light	0.31 ± 0.06	−5.7 ± 0.3	1.7 ± 0.3
Darkness	0.80 ± 0.13	−4.7 ± 0.3	2.3 ± 0.3
High N			
Light	1.02 ± 0.19	−4.7 ± 0.2	2.5 ± 0.2
Darkness	1.00 ± 0.18	−4.2 ± 0.2	2.8 ± 0.2

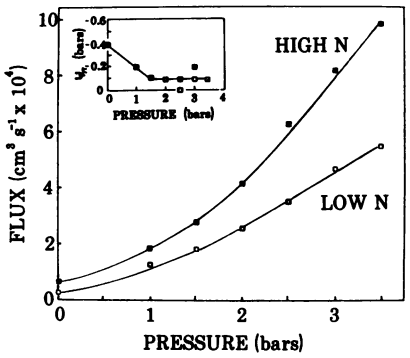

FIG. 2. Water transport through sunflower root systems incubated in a pressure chamber. Rates were determined first at the highest pressure, followed by stepwise decreases in applied pressure. Roots of high N and low N plants had lengths within 20% and areas within 7% of each other. Insert, Osmotic potential of exuded sap.

N deficiency also increased diurnal fluctuations in water potential and turgor of expanding leaves. These fluctuations were in concert with changes in growth rate (Table I). Although the difference in daytime water potential of high N and low N leaves was only 1 bar, it seemed quite likely that altered water relations could have accounted for the growth effects of N.

Hydraulic Conductivities. Cycling of growth (Fig. 1) is typical of mildly water-deficient plants (2, 7) and suggests that in low N plants water is unable to move into the growing leaf cells as easily as in high N plants. Possible differences in water transport properties of the plants were tested using two different methods. In the first, plants were detopped and pressure was applied to the root systems to increase their exudation rate. At both N levels, pressure-induced exudation rate conformed to the general equation (10):

$$J = L'_r (\Delta P + \sigma \Delta \psi_\pi) \quad (5)$$

in which J is the water flux ($cm^3\ s^{-1}$), L'_r is the apparent hydraulic conductivity of the root system ($cm^3\ s^{-1}\ bar^{-1}$), ΔP is the pressure differential between soil and xylem exudate (bars), $\Delta\psi_\pi$ is the osmotic potential differential (bars), and σ is the reflection coefficient (dimensionless). At applied pressures of 1.5 bars or greater, $\Delta\psi_\pi$ became constant (Fig. 2, insert), and the slopes of J against P could be used as measures of L'_r. From this criterion, L'_r of high N root systems was about twice that of low N root systems, 3.8 *versus* 2.0×10^{-4} $cm^3\ s^{-1}\ bar^{-1}$ (Fig. 2). This difference could not be ascribed to gross aspects of root morphology, because measurable differences in root characteristics (especially surface area) were much less than the difference in L'_r.

Apparent hydraulic conductivities of whole plants were estimated from water fluxes into leaves during growth, and the water potential differences between the leaves and soil when no transpiration was occurring. Transpiration was prevented by placing the plants in darkness in an atmosphere saturated with water vapor. Under these conditions, low N leaves elongated 14% slower than high N leaves but displayed a 10% greater water potential difference between leaves and soil (Table II). The L' of high N plants was again about twice that of low N plants, 5.3 *versus* 2.9 $\times 10^{-7}$ $cm^3\ s^{-1}\ bar^{-1}$ (Table II).

Leaf Water Potentials and Growth. Leaf expansion and ψ_w of the expanding blades were followed in high N and low N plants after watering was discontinued. Initial growth rates (well-watered plants) in the light were much greater in high N plants than in low N plants. Initial leaf water potentials were about −4 and −5 bars for high and low N leaves, respectively (Fig. 3). As ψ_w decreased, growth rates dropped rapidly, reaching zero at about −7.5 and −6 bars for high and low N plants, respectively (Fig. 3).

Although N nutrition altered the relationship between growth and ψ_w in the light, it did not change the relationship between growth and turgor (Fig. 3, insert). Within the limits of experimental error, N had no effect upon either m (the slope of the curve) or Y (the threshold turgor for growth). Rather, effects of N apparently resulted mostly from differences in the turgor of the expanding blades, since low N leaves had lower turgor and lower growth rates than high N leaves when the plants were supplied with adequate water. Similarly, growth under nighttime conditions was extremely sensitive to ψ_w, declining rapidly during drying and reaching zero at about −7.5 and −6 bars for high N and low N plants, respectively (Fig. 4). In this case, though, both the maxi-

Table II. *Growth Rates, Growth-Induced Water Potentials, and Hydraulic Conductivities of Expanding Sunflower Leaves with Negligible Transpiration*

Column B represents a coefficient which converts growth rates into water fluxes associated with that growth. Hydraulic conductivities were calculated as A × B ÷ C. Soil water potentials were assumed to be zero. In columns A and C, values are shown ± SE.

Treatment	(A) Growth Rate	(B) dW/dl	(C) Water Potential	Hydraulic Conductivity
	$cm\ s^{-1} \times 10^5$	cm^2	*bars*	$cm^3\ s^{-1}\ bar^{-1} \times 10^7$
Low N	1.17 ± 0.04	0.082	−3.3 ± 0.1	2.9
High N	1.36 ± 0.06	0.117	−3.0 ± 0.2	5.3

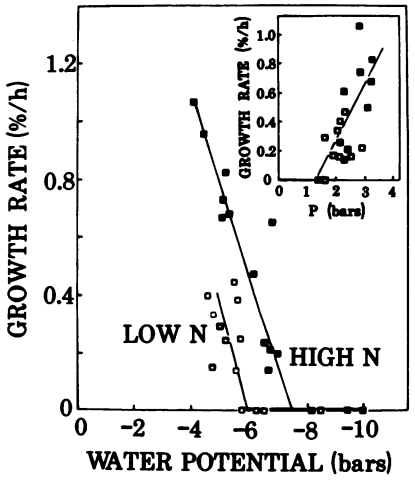

FIG. 3. Growth rates (% increase in length/h) of leaves of sunflower plants grown at two levels of N nutrition, then allowed to dehydrate by withholding water from the soil. Rates were measured at 30°C in the light. Insert, Linear regression of growth rate against turgor pressure ($r = 0.699$).

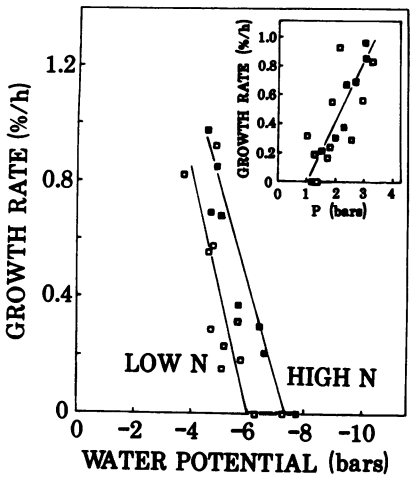

FIG. 4. Growth rates (% increase in length/h) of leaves of sunflower plants grown at two levels of N nutrition, then allowed to dehydrate by withholding water from the soil. Rates were measured at 22°C in darkness. Insert, Linear regression of growth rate against turgor pressure ($r = 0.751$).

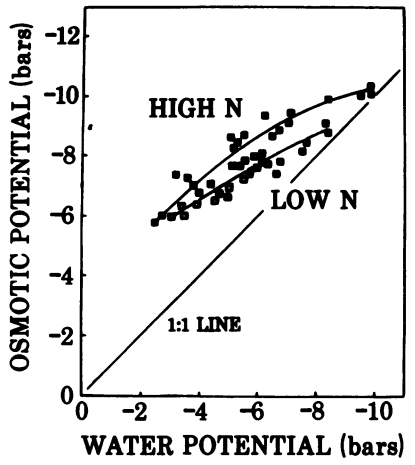

FIG. 5. Relationship between water potential and osmotic potential of expanding sunflower leaves. All points with a water potential <−4 bars (high N) or ≤−4.5 bars (low N) were obtained from plants in the light. Points at a water potential higher than those values were obtained from plants incubated in a dark humid container as described in the text.

mum growth rate and the maximum turgor of low N plants were nearly the same as those of high N plants (Fig. 4). Plots of growth rate against turgor again showed no apparent effects of N nutrition or nighttime conditions on m or Y (Fig. 4, insert; compare to Fig. 3, insert).

Leaf Water Potentials and Osmotic Potentials. N nutrition affected the relationship between ψ_w and ψ_π. Osmotic potentials were consistently lower in high N than in low N plants (Fig. 5). The distance above the 1:1 line in Figure 5 represents leaf turgor. Clearly, low N leaves had lower turgor over the entire range of water potentials in the light, whereas turgor became similar at water potentials achieved in the dark humid chamber (Fig. 5). Presumably differences in cell solute content, or wall elasticity, or both, could account for the differences in osmotic behavior. Preliminary data (not shown) suggested that osmotic adjustment at low water potentials was unaffected by N nutrition. Earlier work with cotton (21) showed that elasticity differences could be important to turgor, but the leaf-to-leaf variability in sunflower precluded any definite conclusions.

DISCUSSION

This study shows that (a) N nutrition altered the water relations of sunflower plants, including the the internal water relations of young expanding leaves; and (b) those changes caused substantial growth inhibition by altering the turgor of expanding leaves during the day. Differences in hydraulic conductivity seemed especially important to growth rates. Because of the low hydraulic conductivity of low N plants, the high transpirational flow during the day caused larger diurnal variations in their leaf water potentials than in high N plants. This effect, combined with the different osmotic potentials at low water potentials, caused greater diurnal changes in turgor in low N than in high N plants. The growth rates of the leaves reflected these changes. Within the limit of experimental error, N nutrition did not affect either wall extensibility (m) or the minimum turgor for growth (Y).

The osmotic potentials and turgors reported here were not corrected for dilution of cell contents by apoplastic water during freezing and thawing. However, values of L' were based upon ψ_w, not ψ_π, and thus were unaffected. Similarly, values of m were obtained from the slope of a curve relating G to P, and an error in estimating P would not change this slope if the magnitude of the error remained more or less constant. Such an error could affect estimates of Y, but earlier work with cotton (21) suggested that N nutrition did not alter the fraction of apoplastic water.

Although N affected both L' (whole-plant) and L'_r (root system), at both N levels L'_r was much greater than L'. Considering only the flux into one second-node leaf, L' (Table II) was about 0.13% of L'_r (Fig. 2) at both N levels. If the flux into the entire growing shoot were used as the basis for comparison (assuming a uniform water potential for all growing tissues), then L' was still only about 1% of L'_r. These data indicate that a limiting resistance to water movement associated with growth was in the shoot rather than the root. Boyer *et al.* (2, 3, 5, 16, 17) have documented the existence of a substantial resistance to water movement into growing cells, and our results are consistent with this model. During the day when water movement through the plant is most rapid, however, most of this flow bypasses the site of highest resistance and does not enter the expanding cells (3). Therefore, daytime water potentials of the expanding leaves were affected primarily by L'_r. Presumably both the root and the shoot conductivities contributed to the overall differences in growth.

It is instructive to compare the magnitudes of L' and m. The slopes of the growth rate-turgor relationships (Figs. 3 and 4, inserts) can be converted to volume units by the coefficient k (see equations 1 and 2). When this is done, km is approximately 5×10^{-7} cm^3 s^{-1} bar^{-1}, or about equal to the L' of high N plants and about twice the L' of low N plants. The algebraic form of equation 4 dictates that growth be more sensitive to the smaller of the two terms (5), in this case L'. Viewed in this light, it is not surprising that the effects of N nutrition on L' altered the growth rate.

The failure of N nutrition to affect m is an interesting observation. Van Volkenburgh and Cleland (25) have recently expanded the 'acid-growth' hypothesis to include leaves. In their experiments, white light stimulated proton transport into cell walls of bean leaves, leading to increased extensibility and initiation of rapid expansion. If proton transport maintains wall extensibility in sunflower leaves, evidently it is unaffected by the alkalinization of the cell interior associated with increased NO_3^- reduction (23). Additionally, wall extensibility was unchanged between light and dark (Figs. 3 and 4, inserts). However, measurements in darkness were made after a preincubation of only about 2 h; quite possibly growth rates may have declined during the 10-h night.

What is the cellular basis for the effects of N on hydraulic conductivity? It is important to note that the low-N and high-N plants had equal leaf areas in our experiments because their ages differed. Root surface areas were also similar. Thus, differences in hydraulic conductivity must have arisen from anatomical, ultra-

structural, or biochemical features. The resistance to water movement across a root is believed to lie largely in cell membranes, and recent evidence suggests that hydraulic conductivity may be related to membrane fluidity, at least in plants at chilling temperatures (13–15). Rivera and Penner (24) found that N deficiency increased the unsaturation level of fatty acids from root cell membranes, but the relationship of N nutrition to membrane properties has not been explored further.

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